FIELD SAMPLING PLAN FOR THE COLLECTION OF OSPREY EGGS FROM THE PORTLAND HARBOR SUPERFUND SITE

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INTRODUCTION

This field sampling plan (FSP) describes the objectives, methods, and procedures for collecting, processing, storing, and shipping eggs from adult osprey (*Pandion haliaetus*) nesting along the Lower Willamette River within and above the Portland Harbor Superfund Site. The specific methods and processing used by analytical laboratories to quantify chemical constituents in eggs, along with the data quality objectives for the analytical methods, will be described in a subsequent Quality Assurance Project Plan (QAPP) to be submitted to EPA prior to osprey egg analysis. This investigation will be conducted as a cooperative effort between the U.S. Environmental Protection Agency (EPA), as part of the Portland Harbor Remedial Investigation/Feasibility Study (RI/FS), and the Portland Harbor Natural Resource Trustees (Trustees) as part of the Natural Resources Damage Assessment (NRDA). Osprey egg tissue will be evaluated to determine baseline contaminant concentrations prior to remedy for the RI/FS, and evaluate potential injury in a top level predator for the NRDA.

BACKGROUND

As top predators, ospreys are good indicators of ecosystem health, and their eggs have been used in the Pacific Northwest and other areas to characterize bioaccumulative contaminants and monitor trends in these chemicals over time (Elliott et al. 1998; 2000, Henny et al. 2003; 2004; 2008). Ospreys along the Willamette River primarily feed on fish (nearly 100% of the diet are fish), and will bioaccumulate organochlorine contaminants into their tissues by consuming contaminated fish prey from waterways close to the nest site (Henny et al. 2003). This process is most important during the month prior to egg laying, when female ospreys are feeding heavily post migration and building their lipid reserves for breeding. These reserves are tapped during egg formation, when mobilized contaminants are available to impact shell thickness and be deposited into the eggs. Elevated concentrations of DDE mobilized in the female osprey during egg development can interfere with supplying calcium to the eggshell gland, resulting in production of eggs with thin shells that are prone to cracking, breaking, and moisture loss which leads to embryo death. Elevated concentrations of other contaminants such as polychlorinated biphenyls (PCBs) in the egg can interfere with growth and survival of the developing embryo. Previously, osprey eggs have been collected within Willamette Basin for contaminant analysis (Henny et al. 2003), but relatively few eggs have been collected from nest sites within the Portland Harbor area.

OBJECTIVES OF OSPREY EGG COLLECTION

The objectives for the collection and analysis of osprey eggs are to:

1. Compare egg health parameters (contaminant concentrations and eggshell thickness) over time in comparison to baseline values established for eggs collected prior to remediation efforts; and
2. Compare egg contaminant concentrations and eggshell thinning to threshold effect and productivity values to better assess injury to the species.

SAMPLING APPROACH

The sampling approach listed will address the following:

- Characterization of concentrations of contaminants of concern in osprey from the Portland Harbor Superfund Site and areas above the site

- Evaluation of productivity of ospreys by aircraft, boat, or surveys

Sample collection will require accessing nest sites by boat, or truck with high-lift equipment, and collection of eggs by hand. One partially incubated egg will be collected per nest. A total of 15 nests will be targeted for collection. Seven nests will be sampled along the lower Willamette River from River Mile (RM) 2.0 to 11 to represent the Portland Harbor superfund study area, one nest will be targeted at RM 19.5, and five nests will be sampled above the falls near Wheatland Ferry (RM 172-180). Productivity and contaminants in eggs from nest sites collected above the superfund site will be used to compare to values within the superfund area. The specific nests sites are as follows:

- RM 2.7 – J.R. Simplot
- RM 3.5 – Head of Multnomah Channel
- RM 5.6 – Cathedral Park Boat Launch
- RM 7.2 – St. John's Railroad Bridge
- RM 8.5 – Swan Island Lagoon, North Slip
- RM 10 – Port of Portland, Terminal 2, North Light Tower
- RM 10.6 – Port of Portland, Terminal 2, South Light Tower
- RM 19.5 – Hospital/Nursing Home
- RM 172-180 Wheatland Ferry Area (five nests to be determined following initial occupancy flight).

Although these nest sites are targeted for egg collection, successfully collecting an egg from each nest will depend on obtaining landowner permission to gain access to the property and the ability to safely climb nest structures.

Productivity surveys will be conducted by air and by boat. Two aerial surveys will be conducted to determine nest activity and final productivity, and any nest sites not evaluated by plane will be evaluated from a boat or vehicle. Productivity will be measured as number of young produced per occupied nest and/or active nest (Postupalsky 1974).

TEAM ORGANIZATION AND RESPONSIBILITIES

Dr. Charles Henny, Dr. Robert Grove and James Kaiser of U.S. Geological Survey (USGS) will coordinate the overall field effort, and Dr. Henny will serve as the Principal Investigator. Dr. Henny, Dr. Grove, James Kaiser and Jeremy Buck of U.S. Fish and Wildlife Service (USFWS) will collect the tissue samples, process the tissue samples in the field or preparation laboratory, and be responsible for sample handling and transport to the field and analytical laboratories.

ROLES AND RESPONSIBILITIES

Dr. Charles Henny,Dr. Robert Grove and James Kaiser:

- Oversee study planning and coordination
- Conduct reconnaissance and identify suitable nest sites for egg collection
- Conduct egg collection and egg processing
- Maintain overwater navigation
- Maintain health and safety of field crew

- Oversee sample transport to field stations, egg preparation, and egg storage
- Maintain eggs in storage until shipment to analytical laboratories

Jeremy Buck

- Oversee tissue collection and processing
- Oversee field collection quality assurance
- Assist in field collections and egg processing

Laboratory manager and Lead chemist/QA manager (identified in QAPP)

- coordinate laboratory analysis
- oversee laboratory
- coordinate data validation

Egg collection and processing will follow this FSP and Oregon Fish and Wildlife Office (OFWO) SOP-F003. OFWO SOP-F003 is attached and incorporated by reference into this FSP. Changes from the FSP or the SOP will be noted on a form and maintained with the project file. Significant changes to the project will be coordinated with EPA Project Managers.

NAVIGATION AND STATION COORDINATES

The location of each nest site targeted for egg collection will be recorded using global positioning system (GPS). The position, time, and date of each sample collected will be recorded.

EQUIPMENT DECONTAMINATION

No decontamination will be needed in the field because eggs will be collected intact and transported to a field laboratory. Each egg will be handled with a new pair of latex or similar gloves to prevent cross contamination among sites. Decontamination of egg processing tools in the field laboratory will follow OFWO SOP-F003.

FIELD DATA MANAGEMENT AND SAMPLE IDENTIFICATION

Information in the field will be clearly recorded on field data sheets and in field notebooks. Information will include personnel, date, time, nest site designation, samples collected, and general observations. Deviations and reasons for deviations from the FSP will be noted in the field book. Fieldbooks will be bound with consecutively numbered pages, and removal of pages will not be permitted. Entries will be made during activities or as soon as possible afterward. Time will follow the 24-hour clock. Corrections to entries will be made with a single line cross out, and corrections will be initialed and dated.

The type of information that may be included in the field logbook and/or field data forms includes the following:

- Names of all field staff
- Sampling vessel
- Station name and location
- Date and collection time of each sample
- Observations made during sample collection, including general weather conditions, complications, and other details associated with the sampling effort

- General description of the collected samples
- Deviations from the FSP or SOP

Sample Identification
A unique code will be assigned to each sample egg. Each sample egg will be labeled according to the river mile where the nest is located. If more than one nest is located within the same river mile, the nest will include an additional letter, e.g., “A,” “B,” etc.

SCHEDULE

Osprey egg sampling will begin approximately May 19, 2008, and will continue for about three days or as needed for up to one week. Two aerial surveys will occur to evaluate productivity; the first will determine occupancy and will occur on May 6 and 7, 2008, and the other will determine final productivity and will occur in late June. Each survey will take approximately 10 hours over a two-day period. Eggs will be submitted to analytical laboratories within 30 days of collection, and analytical results will be received approximately 120 days from submittal.

HEALTH AND SAFETY

The principal investigator will maintain health and safety of field crew. Standard boat operation safety procedures will be followed, and the boat Captain will advise field crew and any new passengers of emergency measures and hazards associated with the vessel prior to launching. All passengers will wear life vests. Climbers accessing nest sites will use safety lines and will wear protective head gear, clothing, and gloves to protect head from falling objects and skin from sharp objects and wood-treating chemicals on treated piers, dolphins, or erected nest-pole structures. Safety glasses will be worn while climbing. Climbers will be attached with a safety line to climbing structure at all times while climbing. Climbing spurs and ascending/descending devices will be used by trained personnel only, and field crew will be advised of hazards associated with climbing spurs and rope use on boats by trained personal. No climbing will be permitted on structures deemed unsafe. Only personnel trained in use of bucket trucks will be permitted to operate buckets to obtain eggs, and assisting field crews will be advised of hazards associated with bucket truck operation.

Only trained personnel will be permitted to open eggs to remove contents, and health and safety concerns associated with sharp instrument use and other hazards will be followed as described in OFWO SOP-F003.

SAMPLING COLLECTION AND PROCESSING

FIELD SAMPLING

Osprey in the lower Willamette River primarily nest on dolphins, pier structures, man-made poles with nest boxes, lighting structures, or electrical towers. Osprey nests will be accessed from a boat or from shoreline. Eggs will be collected by hand after climbing nest structures or accessing elevated nest platforms from a bucket truck. Only structures deemed safe and accessible will be climbed, and electrical towers will not be accessed. One egg per nest will be randomly selected and collected by the climber. Collectors will use latex or similar gloves when handling eggs. Eggs will be placed in a padded can or box and lowered or carried into the boat or to the ground. Samples will be placed on ice in a cooler and transported to the preparation field laboratory at the USGS, Forest and Rangeland Ecosystem Science Center in Corvallis, Oregon, where they will be stored under refrigeration until processing. Egg processing will begin after all egg samples are collected, and will follow specific procedures identified in OFWO SOP-F003.

FIELD LAB PROCESSING
In the field laboratory, eggs will measured and contents harvested as described in OFWO SOP-F003. Egg contents will be placed in chemically-clean jars and frozen at -14°C until shipment to analytical laboratories. Eggshells will be rinsed and set aside to dry for 30 days. Eggshell measurements will then be made according to OFWO SOP-F003.

Nearly all utensils contacting the egg contents during processing will be new and chemically-cleaned. Re-useable utensils, such as probes and spatulas, will be decontaminated as follows.
1. Rinse with potable water.
2. Wash with brush and Alconox™ or other phosphate-free detergent.
3. Rinse twice with deionized water.
4. Rinse with 0.1 N nitric acid.
5. Rinse with 99.5 % acetone.
6. Rinse with deionized water.

All work surfaces where egg processing will take place will be covered with aluminum foil.

Chain-of-Custody Procedures
Samples are in custody if they are in the custodian's view, stored in a secure place with restricted access, or placed in a container secured with custody seals. Each person who has custody of the samples will sign a chain-of-custody record, which will accompany the samples at all times. Chain-of-custody forms will be completed in triplicate; the person responsible for sample collection will retain a copy, and the other two copies will accompany the shipment to the laboratory. Copies of the chain-of-custody forms will be included in laboratory reports. At minimum, the chain-of-custody form will include the following information:

- Site name
- Field coordinators name
- Collection date and time
- Sample type
- Sampling station location
- Number of sample containers shipped
- Requested analyses
- Sample preservation information
- Name of the field coordinator or designee relinquishing the samples to the transporter, noting date and time of transfer and the designated sample custodian at the receiving facility
- Signature, date, time of receipt by custodian at receiving facility

The field coordinator or a designated field sample custodian will be responsible for all sample tracking and chain-of-custody procedures for samples in the field. The sample custodian will be responsible for final sample inventory and will maintain sample custody documentation. The custodian will complete chain-of-custody forms prior to removing samples from the sampling vessel. Upon transferring samples to the laboratory sample custodian, the field sample custodian will sign, date, and note the time of transfer on the chain-of-custody form.

The original chain-of-custody form will be transported with the samples to the laboratory. The laboratory sample custodian will be responsible for receiving samples and logging them into the laboratory's tracking system. The custodian will ensure that the chain-of-custody and sample tracking forms are properly completed, signed, and initialed upon transfer of the samples.
The custodian will enter the sample number into a laboratory tracking system by project code and sample designation.

When all samples have been packed, the chain-of-custody form will be placed into a zip-lock bag and taped on the inside lid of the cooler. Sample shipment will follow OFWO SOP-F003.

QUALITY CONTROL PROCEDURES

Field quality control (QC) samples typically include replicates to evaluate variability, splits to evaluate sample homogenization, or temperature blanks evaluate storage conditions. This study will evaluate a replicate of one egg that will be split at the analytical laboratory by dividing the homogenate and analyzed as separate samples. Other field QC measures are not practical due to the sample type, or not deemed necessary.

CHEMICAL ANALYSIS

Osprey egg tissue samples will be analyzed for the following:

- Polybrominated diphenyl ethers (PBDEs) (15 congeners including PBDE209)
- Polychlorinated biphenyl (PCB) congeners (40 congeners which include about 95% of what is typically present in osprey eggs)
- Dioxins and furans
- Organochlorine pesticides
- Mercury
- Lipid content
- Percent moisture

REPORTING

A brief written update summarizing success of field sampling efforts will be submitted to EPA and the Trustees within 60 days of completion of field efforts. The update will include a summary of sample locations and number of eggs collected, egg processing results, and status of project.

REFERENCES

Elliott, J.E., M.M. Machmer, C.J. Henny, L.K. Wilson, and R.J. Norstrom. 1998. Contaminants in ospreys from the Pacific Northwest: I. Trends and patterns in polychlorinated dibenzo-p-dioxins and -dibenzofurans in eggs and plasma. Archives of Environmental Contamination and Toxicology 35(4):620-631.

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Oregon Fish and Wildlife SOP-F003

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Bird Egg Collection, Measurement, Preparation, and Shipment for Residue Analysis

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Background and Objectives

Environmental contaminants can be transferred into eggs from adult female birds at concentrations that can be detrimental to the developing embryo. Contaminants can directly impact the embryo, or cause eggshell thinning which can dehydrate the embryo or lead to egg breakage during incubation. Collection of bird eggs is useful in wildlife toxicology to evaluate contaminant concentrations in egg contents, and measure the degree for eggshell thinning, to monitor trends over time or evaluate risk from contaminant exposure to the species. The objectives of this Standard Operating Procedure (SOP) are to 1) provide a consistent method for egg collection; 2) ensure consistent measurements of the egg and provide standard methods to measure eggshell thickness; and 3) ensure accurate analysis of contaminants in eggs by providing standard methods for harvesting and transferring egg contents into a clean container without introducing contamination. It is important to standardize the process for collecting eggs, harvesting egg contents, and measuring eggshell thickness to improve data comparisons among contaminant investigations. Collection of various measurements during egg processing is necessary for interpretation of analytical results.

Materials

For field collection: Appropriate State and Federal permits; waterproof pen or pencil; specimen jar labels; egg collection cans or boxes (padded coffee can, hard-sided container such as plastic kitchen ware, or tackle box with foam padding); aluminum foil; small plastic bags with zip closure.

For egg processing in laboratory: Data sheets; writing utensils; dull pencil; safety glasses; powder-free latex gloves; laboratory paper wipes such as Kimwipes®; distilled, deionized (DDI) water or equivalently pure water; balance (to 0.01 g); vernier calipers (to 0.01 mm); immersion chamber with beaker and wire loops (Figure 1) or similar vessel to measure egg volume; chemically-clean jar (one per egg); chemically-clean stainless steel scalpel blades (No. 21 or No. 22 with No. 4 handles or similar size); chemically-clean aluminum foil sheets (approximately 30 x 30 cm square), 1 per egg; ball-tip micrometer (to 0.01 mm).

Collection

The procedure for accessing the nest and determining which egg to collect, and how many eggs, will vary depending on the species and study objectives. Typically, one egg per nest is randomly selected and removed.

1. Remove egg(s) from nest. Wrap egg in chemically-clean aluminum foil - dull dull side in - if needed (aluminum foil keeps the eggshell together and the contents inside should the egg be cracked in transit), and/or place egg in zip-closure plastic bag. Clearly label egg in pencil or place label (written in pen) in plastic bag with egg. Include on label the date, collector, nest identification, and location. Place protective material around egg (e.g., gently wrap egg in bubble wrap, place in foam rubber in which egg holes have been cut, or place in an egg carton).

2. Place each egg in container (a clean 1-gallon or quart paint can, cardboard box, Tuperware container, etc.) and fill spaces with soft packing material. Seal container with tape.

3. Pack the egg container inside ice chest with blue ice (to maintain 4EC temperature) and cushion with appropriate amount of packaging material.

4. If eggs cannot be processed immediately after collection, store eggs in a refrigerator (4EC). ***Do not freeze*** whole eggs since this will crack the shell.

<u>Egg Processing</u>

Whole Egg Measurements

1. If possible, candle the egg to determine if cracks are present in the shell. Any cracked egg should not be rinsed or immersed in water as this may contaminate the sample.

2. If the egg is not cracked and is dirty, clean gently with a soft towel and distilled or deionized water that is at or near the temperature of the egg. Dry the egg.

3. Write the sample identification number on both ends of the eggshell with a dull pencil.

4. Record on data sheet any distinguishing characteristics of the egg (e.g. cracked, dented, discolorations, etc.).

5. Measure and record the length (mm) [caliper jaws parallel to the longitudinal axis] and the breadth (mm) [caliper jaws perpendicular to the longitudinal axis] of the egg with calipers at their greatest dimensions.

6. Measure and record the mass (g) of the egg on data sheet.

7. Measure and record the egg volume (cm^3) by following instructions given below for intact versus cracked shells. NOTE: Egg volume is important for estimating lipid and moisture loss of an egg, and is used along with other eggshell metrics to convert analytical results from wet weight to fresh weight (which incorporates lipid and moisture loss). If egg is cracked, do not immerse it in water; rather, see Cracked Egg Technique below.

Intact Shell-Water Displacement Technique

1. Place a water receptacle adjacent and underneath the egg immersion volumeter side arm (see Figure 1).

2. Place the wire egg holder in the volumeter.

3. Fill the volumeter with distilled or deionized water until it flows freely from the volumeter side arm (*Note: the temperature of the water should be as close to the temperature of the egg as possible*).

4. When the water stops flowing, the receptacle should be emptied, weighed, and returned to its position adjacent to the volumeter.

5. Gently raise the wire egg holder and place the egg on it. Gently lower the egg into the volumeter until it is completely submersed.

6. Weigh the water receptacle and its contents. Subtract the mass of the water receptacle alone. The mass of the displaced water is the approximate egg volume, assuming that egg density is similar to water (1gm = 1 ml). For example, 40 gm displaced water = 40 ml of water, and 40 ml egg volume.

7. Repeat the procedure 3 times for each egg and report the average value. Dry the egg.

8. Calculate the fresh weight conversion factor and record value on data sheet. Use the equation:

$$\text{Conversion factor} = \frac{\text{egg contents mass (g)}}{\text{displaced water mass (g)}}$$

Alternative method for measuring volume:

1. Place egg in a graduated cylinder with 10 ml graduations.

2. Adjust water level to a given graduation, then immerse the egg with a wire egg holder.

3. Determine the first volume of water displaced in complete 10 ml units. Then, use a 10 ml syringe to collect the water in the graduated cylinder down to the nearest 10 ml graduation. Measure the difference in water volume as determined on the graduated cylinder.

4. Add the amount of water in the 10 ml syringe (measured in 0.1 ml increments) e.g., 50 ml + 5.2 ml = volume of egg, 55.2 ml.

Cracked Egg Technique

Do not measure volume using egg immersion. Instead, estimate egg volume based on length and breadth measurements, depending on species, as described in Stickel *et al*. (1973):

Embryo Harvest

Wear surgical gloves and safety glasses when performing embryo harvest. **CAUTION: EGGS MAY EXPLODE UPON OPENING IF CONTENTS HAVE DECOMPOSED!**

1. If eggs have a strong odor (indicating advanced decomposition) or are suspected to be addled, it is advisable to vent before attempting to open to avoid possible explosions. To vent, don safety glasses and gently insert a chemically-clean needle into the blunt end of the egg. Use gentle, steady pressure to pierce the shell.

2. Place a labeled, chemically-clean glass jar and its lid on a scale, tare, and remove lid. Jars should be labeled with the following information; sample number, species, date, location, and collector. An additional label containing the sample number and date should be placed on the lid of each jar.

3. Hold the egg lengthwise over the jar. Using a sharp scalpel, gently score the egg around its equator. Apply gentle, steady pressure while making several rotations around the egg. Once the scalpel pops through the shell, insert the tip of the scalpel blade to cut the membrane and separate the two halves. Cut 1/2 to 2/3 the distance around the egg. Invert the egg while pulling apart the shell halves and pour the contents into the opened jar. Use a chemically clean stainless steel or teflon spatula to scrape any contents remaining on the shell into the jar (be careful not to tear the shell membrane).

4. Weigh (g) the jar (including lid) containing the egg contents. Record the mass (g) of the egg contents by subtracting the mass of the jar and lid alone from the jar and lid containing the egg contents.

5. Visually inspect the egg contents. Record the presence or absence of an embryo, estimated stage of development as early, mid, or late. Note any abnormalities.

6. Rinse the interior of the shell halves with tap water being careful not the tear the membrane, or erase the sample identifications. After the shells have dried, use a indelible ink pen to remark the shells with their sample IDs. Store the shells in a cool dry place for at least 30 days at which time they should have attained a constant mass. Store egg shells in egg cartons.

7. Store embryos in freezer at -13EC.

Shell Thickness Measurement

1. Determine the eggshell mass or weight (to the nearest 0.001 g) of dried shells.

2. Measure the eggshell thickness using a dial micrometer with rounded contacts. Take thickness measurements of each shell-half along the equator in at least three places. Report the average of all measurements as the final thickness measurement. If the membrane has separated from the shell, take measurements without the membrane but be sure to make note of this on the data sheet.

3. If determining the Ratcliff thickness Index (Ratcliffe 1967), calculate using the following formula:

$$\text{Thickness Index} = \frac{\text{eggshell mass (mg)}}{\text{egg length (mm) X egg width (mm)}}$$

Sample Shipment

1. Wrap sample jars with bubble wrap and secure with tape or parafilm.

2. Place samples in cooler with a sufficient supply of dry ice (1 gm dry ice: 1 gm sample) to last at least 24 hours.

3. Cushion samples in cooler with packing material such as foam rubber, bubble wrap, or peanuts.

4. Enclose the working catalog, a pre-addressed return label, and chain of custody forms in a sealed plastic bag inside cooler.

5. Wrap cooler with mailing tape.

6. If needed, inform the shipper that the cooler contains dry ice by labeling two opposite sides and the top of the cooler with a dry ice label containing the amount of dry-ice inside.

7. Call the designated laboratory contact to inform him/her that samples have been shipped.

References

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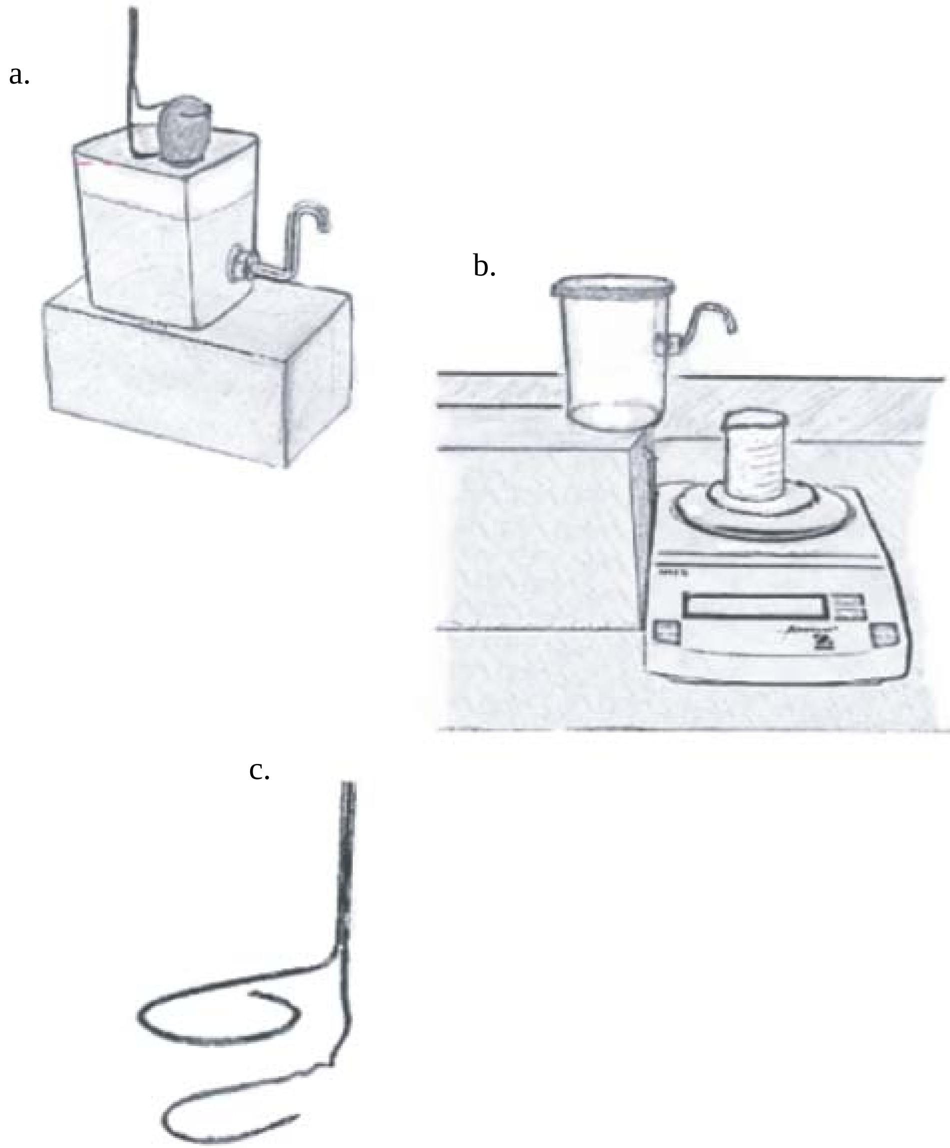

Figure 1. Measuring total egg volume. a. Egg immersion chamber (can be round or square shaped). The top bend of the spigot is high enough so that an egg can be completely immersed below it. b. Immersion chamber set up to drain into beaker on balance. c. Wire loops used to hold the egg.